

In alternative preferred embodiments, qualitative or quantitative amplification for EGFr mRNA or cDNA, including EGFr mRNA or cDNA corresponding to a mutated or altered EGFr gene, is performed by methods known to the art, *for example*, methods described by LeRiche *et al.* (1996, *J. Clin. Endocrinol. Metab.* 81: 656-662), Dahiya *et al.* (1996, *Urology* 48: 963-970), 5 Schlegel *et al.* (1994, *Int. J. Cancer* 56: 72-77), and Worm *et al.* (1999, *Hum. Pathol.* 30: 222-227), these references herein incorporated by reference in their entirety.

In a preferred embodiment, PCR amplification of her-2/neu cDNA is performed by the method of Pawlowski *et al.* (2000, *Cancer Detection Prev.* 24: 212-223), herein incorporated by reference in its entirety, adapted as follows. A PCR reaction mixture is prepared in a 50 microL final volume containing 5 microL cDNA, 1.5 mM MgCl<sub>2</sub>, 0.8 mM of each dNTP, 2 Units Taq DNA polymerase (Eurobio, Les Ulis, France), and 0.4 microM each her-2/neu primers (SEQ ID Nos. 8 and 9). PCR is performed in a thermocycler for 45 cycles under a temperature profile consisting of an initial denaturation at 94 degrees C for 5 minutes, followed by denaturation at 94 degrees C for 30 seconds, annealing at 60 degrees C for 20 seconds, and extension at 72 degrees C for 60 seconds, with a final extension at 72 degrees C for 8 minutes. Detection of the amplified product is achieved, for example by gel electrophoresis through a 1.5% agarose gel, using ethidium bromide staining for visualization and identification of the product fragment.

In alternative preferred embodiments, qualitative or quantitative amplification for her-2/neu mRNA or cDNA is performed by other methods known to the art, *for example*, methods as 20 described by Pawlowski *et al.* (2000, *Cancer Detection Prev.* 24: 212-223) for real-time quantitative RT-PCR, Walch *et al.* (2001, *Lab. Invest.* 81: 791-801), Sarkar *et al.* (1993, *Diagn Mol. Pathol.* 2: 210-218), Gebhardt *et al.* (1998, *Biochem. Biophys. Res. Comm.* 247: 319-323),

Revillion *et al.* (1997, *Clin. Chem.* 43: 2114-2120), or Schneeberger *et al.* (1996, *Anticancer Res.* 16: 849-852), these references incorporated herein by reference in their entirety.

In a preferred embodiment, RT-PCR for c-myc mRNA is performed by the method of Kraehn *et al.* (2001, *Br. J. Cancer* 84: 72-79), incorporated herein by reference in its entirety, 5 using 5 microliters of c-myc cDNA in a PCR reaction mixture containing PCR buffer, 1.5 mM Mg<sup>2+</sup>, 0.2 mM each dNTP, 1.7 Units Taq polymerase (Boehringer, Mannheim, Germany), and 0.5 microM each c-myc primer (SEQ ID Nos. 13 and 14, identified above). The mixture is amplified in a thermocycler under a temperature profile consisting of an initial 4 minute denaturation at 94 degrees C, followed by 45 cycles of denaturation at 93 degrees C for 35 seconds, annealing at 60 degrees C for 35 seconds, and extension at 72 degrees C for 35 seconds, followed by a final extension at 68 degrees C for 10 minutes. Detection of the amplified product is achieved, for example by gel electrophoresis through a 2% agarose gel, using ethidium bromide staining for visualization and identification of the amplified product.

In alternative preferred embodiments, qualitative or quantitative amplification for c-myc mRNA or cDNA is performed by other methods known to the art, *for example*, methods described by Gamberi *et al.* (1998, *Oncology* 55: 556-563), Sagawa *et al.* (2001, *Cancer Letters* 168: 45-50), Christoph *et al.* (1999, *Int. J. Cancer* 84: 169-173), and Latil *et al.* (2000. *Int. J. Cancer* 89: 172-176), these references incorporated herein by reference in their entirety.

In a preferred embodiment, RT-PCR for hnRNP A2/B1 RNA is performed by the method of Zhou *et al.* (1996, *J. Biol. Chem.* 271: 10760-10766), incorporated herein by reference in its entirety, but wherein 5 microliters of cDNA is used in the reaction mixture, and the PCR amplification is performed for 45 cycles. The amplified product is then detected by gel electrophoresis through a 2% agarose gel using ethidium bromide staining for visualization and

identification of the product. In alternative preferred embodiments, qualitative or quantitative amplification of hnRNP A2/B1 RNA, or associated RNA such as hnRNP A2 RNA or hnRNP B1 RNA is performed by other methods known to the art, *for example*, methods described by Kozu *et al.* (1995, *Genomics* 25: 365-371), incorporated by reference herein in its entirety.

5 In alternative preferred embodiments, amplified EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 RNA or any combination thereof or cDNA products thereof can be detected using methods, including but not limited to other gel electrophoresis methods; capillary electrophoresis; ELISA or modifications thereof, such as amplification using biotinylated or otherwise modified primers; nucleic acid hybridization using specific, detectably-labeled probes, such as fluorescent-, radioisotope-, or chromogenically-labeled probes; Southern blot analysis; Northern blot analysis; electrochemiluminescence; laser-induced fluorescence; reverse dot blot detection; and high-performance liquid chromatography. Furthermore, amplified product fragment detection may be performed in either a qualitative or quantitative fashion.

PCR product fragments produced using the methods of the invention can be further cloned into recombinant DNA replication vectors using standard techniques. RNA can be produced from cloned PCR products, and in some instances the RNA expressed thereby, *for example*, using the TnT Quick Coupled Transcription/Translation kit (Promega, Madison, Wisconsin) as directed by the manufacturer.

In another embodiment, restriction digestion may be performed upon a single-stage RT-PCR product. The products of the restriction digestion can be further amplified in a second stage amplification reaction using appropriate primers.

In a preferred embodiment, EGF, EGFr, her-2/neu, c-myc, or hnRNP A2/B1 RNA or any combination thereof or cDNA produced therefrom is amplified in a quantitative fashion thereby